

O-25 - ASHBYA GOSSYPII RIBOFLAVIN OVERPRODUCING STRAINS ARE HIGHLY SUSCEPTIBLE TO LIGHT-INDUCED OXIDATIVE DNA DAMAGE

Rui Silva¹; Tatiana Q. Aguiar¹; Rui Oliveira²; Lucília Domingues¹

1 - CEB – Centre of Biological Engineering, University of Minho, 4710-057 Braga, Portugal; 2 - CITAB – Centre for the Research and Technology of Agro-Environmental and Biological Sciences, Department of Biology, University of Minho, 4710-057 Braga, Portugal

Background

The overproduction of riboflavin by *Ashbya gossypii*, which is one of the most distinctive traits of this filamentous hemiascomycete, is triggered by oxidative stress [1]. In turn, riboflavin is a strong photosensitizer that upon irradiation with light has been shown to generate reactive oxygen species (ROS) and induce oxidative DNA damage in mammalian cells [2-3]. Envisioning a better understanding of this *A. gossypii* trait, here we investigated whether riboflavin overproduction is associated with increased DNA damage.

Method

The DNA damage accumulation in riboflavin overproducing and non-overproducing *A. gossypii* wild strains was assessed with a newly developed *Ashbya* Comet Assay (Single Cell Alkaline Gel Electrophoresis). This protocol is an adapted and optimized version of the Yeast Comet Assay [4] and is here shown to reproducibly measure oxidative (H₂O₂/menadione-mediated) and non-oxidative (camptothecin-mediated) DNA damage in *A. gossypii*. Radial growth and riboflavin production was assessed on agar-solidified AFM after incubation in the dark or under a visible fluorescent lamp for three days.

Results & Conclusions

The newly developed *Ashbya* Comet Assay allowed the reproducible measurement of H₂O₂/menadione-mediated (oxidative) and camptothecin-mediated (non-oxidative) DNA damage. Further assessment of the DNA damage in different *A. gossypii* wild strains with this validated protocol revealed significantly higher DNA damage accumulation in the riboflavin overproducing strain when it was exposed to light during growth. However, no significant differences were observed in terms of growth or riboflavin production by this strain. The non-overproducing strain did not display significant differences between conditions in any of the measured parameters. These evidences show that the accumulation of riboflavin in *A. gossypii* makes it highly susceptible to light-induced oxidative DNA damage, similarly to what occurs in mammalian cells [2-3]. These results thus draw attention for the importance of controlling the exposure to light of biotechnological riboflavin production processes (with *A. gossypii* or other organisms).

Acknowledgments

Study supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UID/BIO/04469/2013 unit and COMPETE 2020 (POCI-01-0145-FEDER-006684) (Post-Doc grant to T. Q. Aguiar), BioTecNorte operation (NORTE-01-0145-FEDER-000004) funded by the European Regional Development Fund under the scope of Norte2020 - Programa Operacional Regional do Norte, Project RECI/BBB-EBI/0179/2012 (FCOMP-01-0124-FEDER-027462) and PhD grant PD/BD/113812/2015 to R. Silva.

References

1. Aguiar *et al.* (2015) *Biotechnol Adv* 33:1774-86.
2. Besaratinia *et al.* (2007) *PNAS* 104:5953-58.
3. Alam *et al.* (2015) *Arch Biochem Biophys* 584:10-9.
4. Azevedo *et al.* (2011) *Yeast* 28:55-61.

Keywords: *Ashbya gossypii*, Riboflavin overproduction, Oxidative stress, DNA damage, Comet assay